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Relationships between the Slope of the Oxygen Equilibrium Curve and the Cooperativity of Hemoglobin as Analyzed Using a Normalized Oxygen Pressure Scale

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ABSTRACT—The oxygen equilibrium curve (OEC) for hemoglobin, which is usually expressed as a S *vs.* P plot, was expressed by normalizing P by P_{dmax}, where S is oxygen saturation, P is partial pressure of oxygen, and P_{dmax} is P at which the slope of the usual OEC is maximized. The maximal slope of normalized OEC gives $P_{dmax} \cdot S'_{max}$, where S'_{max} is the maximal slope of the usual OEC, a measure for the oxygen transport efficiency of hemoglobin. Here, the term "efficiency" is used in the sense that the oxygen release from hemoglobin becomes more sensitive to oxygen pressure changes as S'_{max} becomes larger. An analysis using 38 sets of published oxygen equilibrium data for human adult hemoglobin under various experimental conditions showed that (a) expressing OEC by means of S *vs.* P/P_{dmax} or S *vs.* log P is advantageous for analyzing the slope of OEC compared to usual S *vs.* P plot and (b) while the OEC differs depending on experimental conditions, S'_{max} varies in close linear correlation to n_{max} (the maximal slope of the Hill plot which measures oxygen binding cooperativity), and $P_{dmax} \cdot S'_{max}$ is almost equal to $n_{max}/4$. Thus, the parameter expressing oxygen transport efficiency is closely related to the parameter expressing oxygen binding cooperativity.

INTRODUCTION

The sigmoid shape of the oxygen equilibrium curve (OEC) of hemoglobin (Hb) is physiological significant since the oxygen release from hemoglobin is sensitively regulated in response to the oxygen demands of tissue. The sigmoid character of OEC is ascribed to cooperative oxygen binding arising from heme-heme interactions. Cooperativity has been conventionally measured by the Hill coefficient, n_{max}, which is defined as the maximal slope of the Hill plot, i.e., log [S/(1-S)] *vs.* log P plot, where S and P are the oxygen saturation of hemoglobin and partial pressure of oxygen, respectively [2, 9]. The entire OEC is well described by the Adair equation [1] as follows:

$$S = \frac{(K_1P+3K_1K_2P^2+3K_1K_2K_3P^3+K_1K_2K_3K_4P^4)}{(1+4K_1P+6K_1K_2P^2+4K_1K_2K_3P^3+K_1K_2K_3K_4P^4)} \quad (1)$$

Here, K_i (i=1 to 4) is the intrinsic equilibrium constant for the ith oxygen binding step (stepwise Adair constant).

The efficiency of oxygen transport by hemoglobin is assessed by the first derivative of S, (S'(=dS/dP)), with respect to P. Plotting S' against P yields a bell-shaped curve. The ordinate and abscissa readings at the top of the curve give the maximal oxygen transport efficiency, S'_{max}, and the partial oxygen pressure at that point, P_{dmax}, respectively. Our recent analysis [6] using 38 sets of published accurate oxygen equilibrium data including Adair constant values showed that S_{nmax} (S at which n_{max} occurs) does not agree with S_{dmax} (S at $P=P_{dmax}$), the latter mostly being 0.38 irrespective of the experimental conditions. This indicates that the point on the OEC at which cooperativity is maximized is not necessarily the same as the point at which the oxygen transport efficiency is maximized. In the previous analysis, P_{dmax} varied widely depending on the overall oxygen affinity, usually measured by P_{50} (oxygen pressure at half oxygen saturation).

In the present paper, we describe a specific way of drawing the OEC in which the abscissa is normalized by P_{dmax}. By using this normalization, the maximal slope of OEC as a measure of oxygen transport efficiency is found to be closely related to oxygen binding cooperativity.

METHODS

The experimental data used for the present analysis were taken from 38 sets of published oxygen equilibrium data for human adult hemoglobin (Hb A) [3, 4, 5, 8] obtained at 25°C under a variety of solutions conditions such as pH (6.5, 7.4 and 9.1), Cl^- concentration (2.6 mM, 7 mM and 100 mM), 2,3-diphosphoglycerate concentration (0 and 2 mM), inositol hexaphosphate concentration (0 and 2 mM) and CO_2 pressure (0 and 40 mmHg). These data included Adair constant values obtained by a nonlinear least-squares curve-fitting method. These Adair constant values were used for calculating various parameters and the construction of curves that appear in the present study.

All computations were carried out on a personal computer model PC-9821 Ap2 using MS-FORTRAN (Nippon Electric Co.,

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RESULTS AND DISCUSSION

Normalization of the OEC abscissa

It was shown in our previous analysis [6] that the S' value strongly depends on the position of the OEC along the abscissa: S'_{max} was inversely related to the parameters expressing the position of OEC, such as P_{dmax} or P_{50} (P at S= 0.5). It is therefore expected that the dependence of S'_{max} on the position of the OEC becomes less distinct if P is made dimensionless by normalizing it with some parameter representing the position of OEC along the abscissa. We used P_{dmax} as this parameter. Figure 1 shows three examples of normalized OEC determined in different solutions. The parameter values for three OECs are listed in Table 1.

TABLE 1. Parameter values for three examples of OEC

	Solution conditions	P_{50} (mmHg)	P_{dmax} (mmHg)	S'_{max} ($mmHg^{-1}$)	n_{max}
OEC A	pH 9.1, 2.6 mM Cl^-	1.04	0.75	0.67	2.35
OEC B	pH 9.1, 0.1 M Cl^-	2.13	1.68	0.36	2.73
OEC C	pH 7.4, 0.1 M Cl^- 5% CO_2	7.84	6.65	0.11	3.15

These normalized OECs are located at roughly similar positions, taking into account that the P_{dmax} value for the 38 OECs varied from 0.75 to 15.48 mmHg. If other OECs are plotted together, they would be close to curve C. This normalization allows us to analyze the shape of OEC in a more direct way.

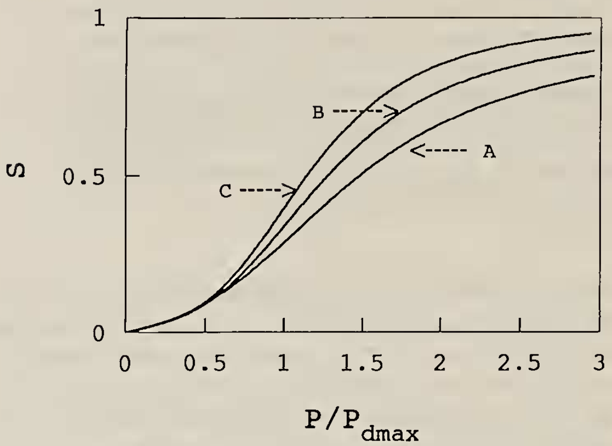

FIG. 1. Oxygen equilibrium curves (OEC) of human Hb A normalized by P_{dmax}. OEC data taken from Imai [3]. S, fractional oxygen saturation; P, partial pressure of oxygen; P_{dmax}, P at which S'(=dS/dP) is maximized. Solution are given in Table 1.

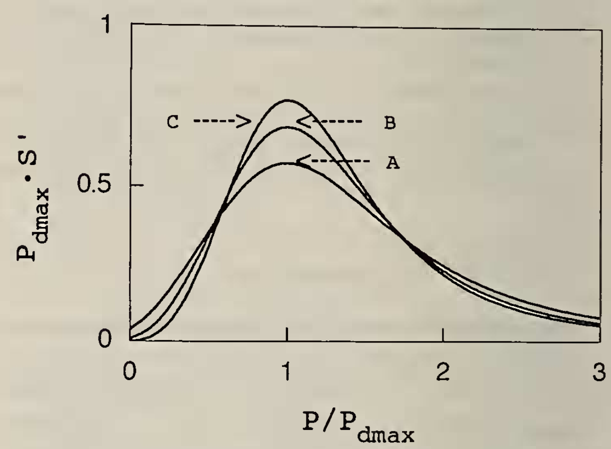

FIG. 2. $P_{dmax} \cdot S'$ *vs.* P/P_{dmax} plots for human Hb A. S' is the first derivative of S with respect to P. The OEC data sets are the same as those in Fig. 1.

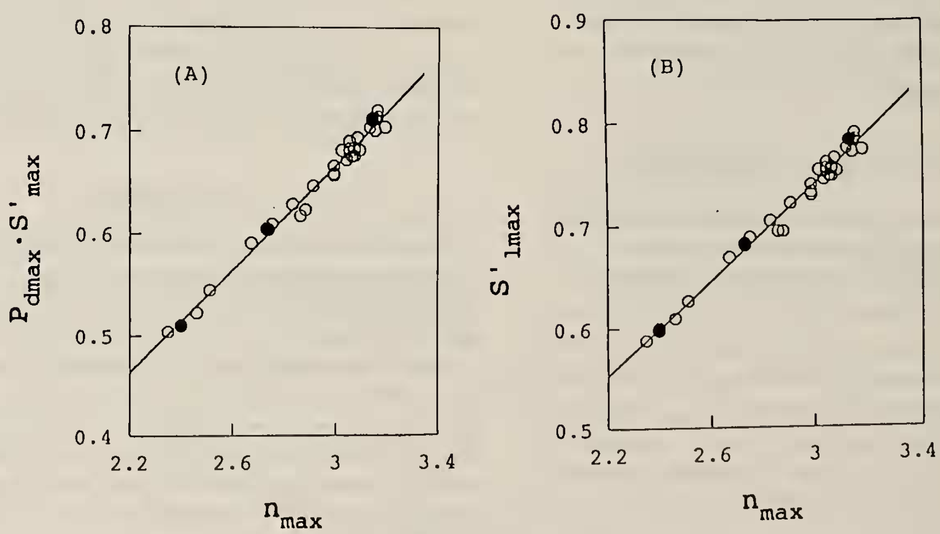

FIG. 3. Correlation between $P_{dmax} \cdot S'_{max}$ and n_{max} (A) and between S'_{lmax} and n_{max} (B) for human Hb A. The $P_{dmax} \cdot S'_{max}$ values were calculated from the slope of normalized OECs at $P/P_{dmax} = 1$ for the 38 OEC data sets [3–5, 8]. S'_{lmax} is the maximal slope of the S *vs.* log P plot (see Fig. 4). The OEC data sets are the same as in Fig. 3 A. Closed circles are the data sets shown in Fig. 1.

Relation between the maximal slope of normalized OEC and n_{max}

The slope of normalized OEC gives the $P_{dmax} \cdot S'$ value. In Figure 2, the same sets of data as in Figure 1 are plotted by means of $P_{dmax} \cdot S'$ *vs.* P/P_{dmax}, yielding bell-shaped curves. As opposed to the previous S' *vs.* P plot [6], the tops of the bell-shaped curves in Figure 2 are located at the same position along the normalized abscissa. At the top where $P/P_{dmax}=1$, the $P_{dmax} \cdot S'$ value is maximized. The higher the top (or the larger the $P_{dmax} \cdot S'_{max}$ value), the larger the n_{max} value.

Figure 3 (A) shows the correlation's between $P_{dmax} \cdot S'_{max}$ and n_{max} for the 38 sets of OEC data. These two parameters are closely correlated, showing a linear regression:

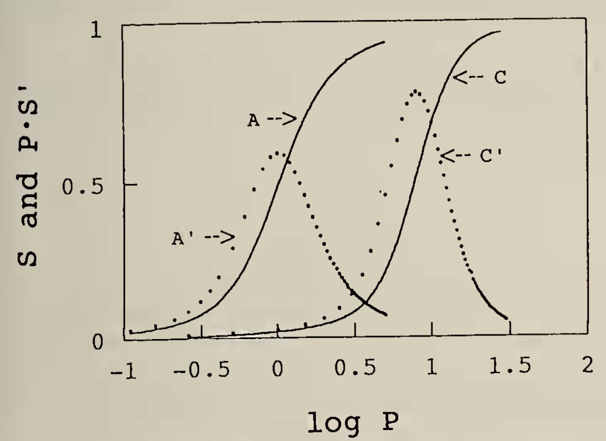

FIG. 4. S *vs.* log P plots (solid lines) and S'·P *vs.* log P plots (dotted lines) of human Hb A. Two of the three OEC data sets in Fig. 1 were used. P·S' was calculated from the slope of the S vs. log P plot (Eq. (3)).

$$P_{dmax} \cdot S'_{max} = 0.25 \cdot n_{max} - 0.09 \ (r=0.98). \quad (2)$$

The coefficient values indicate that $P_{dmax} \cdot S'_{max}$ is roughly equal to 1/4 of n_{max} and the maximal slope of the normalized OEC is closely related to oxygen binding cooperativity.

Relation between the maximal slope of the S vs. log P plot and n_{max}

Expressions of OEC by means of a S *vs.* log P plot are advantageous in the sense that changes in overall oxygen affinity are converted to parallel displacement of OEC along the abscissa and comparison of the shape is easy.

Figure 4 shows two sets of OEC data chosen from the three sets in Figure 1 as expressed by S *vs.* log P and their slope as a function of log P. Since

$$dS/d\log P = P \cdot dS/dP = P \cdot S', \quad (3)$$

$P_{dmax} \cdot S'_{max}$ can be calculated from the slope of the S *vs.* log P plot at $P=P_{dmax}$, yielding the same linear regression as Eq. (2). The maximal slope (S'_{lmax}) of the S *vs.* log P plot also yielded a similar linear regression:

$$S'_{lmax} = 0.23 \cdot n_{max} + 0.04 \ (r=0.98) \quad (4)$$

(see Fig. 3(B)). Interestingly, the maximal slope occurred in the narrow range of S: S=0.49 to 0.54. or at $S=0.52 \pm 0.01$SD for all 38 data sets.

Eqs. (2) and (4) indicate that $P_{dmax} \cdot S'_{max}$ is related to S'_{lmax}. In fact, these two quantities showed a very close correlation:

$$P_{dmax} \cdot S'_{max} = 1.08 \cdot S'_{lmax} - 0.133 \ (r=0.999). \quad (5)$$

Since $P_{dmax} \cdot S'_{max}$ varied from 0.5 to 0.7 while S'_{lmax} varied from 0.6 to 0.8 depending on the solutions, the latter can be a rough approximation for the former. Thus, the maximal slope of the S *vs.* log P plot gives basically the same information as that of the S *vs.* P/P_{dmax} plot. The constant term of the right-hand side of Eq. (5) originates from the constant terms in Eqs. (2) and (4). There is no theoretical necessity for these 3 terms to be zero since these 3 equations are empirical.

Basis for the relation between $P_{dmax} \cdot S'_{max}$ *and* n_{max} *and its significance*

It holds that

$$n = d\log [S/(1\text{-}S)]/d\log P$$
$$= [(1/(S(1\text{-}S))] \cdot dS/d\log P \quad (6)$$

where n is the slope of the Hill plot at a given P value [8]. From Eqs. (3) and (6) it follows that

$$P \cdot S' = S(1\text{-}S) \cdot n. \quad (7)$$

At $P=P_{dmax}$, the S values for the 38 OEC data sets are approximately equal to 0.38 [4] and n is somewhat smaller than n_{max}. Thus, $P_{dmax} \cdot S'_{max} \simeq 0.24 \cdot n_{max} \simeq n_{max}/4$. As described above, S'_{lmax} is roughly equal to $P_{dmax} \cdot S'_{max}$, and the former occurs around S=0.5. Furthermore, n at S=0.5 is only insignificantly smaller than n_{max}. Therefore, $P_{dmax} \cdot S'_{max} \simeq 0.5(1-0.5) \cdot n_{max} = n_{max}/4$. These approximations would be more concise if the OEC expressed by S *vs.* log P was more symmetric and thereby the values of P_{dmax}, P_{50} and P_{nmax} (at which n_{max} occurs) were closer to each other.

The present analysis has shown that (a) the expression of OEC by means of S *vs.* P/P_{dmax} or S *vs.* log P is advantageous for analyzing the slope of OEC compared to a conventional S *vs.* P plot and (b) S'_{max} is closely related to n_{max}, while $P_{dmax} \cdot S'_{max}$ is approximately equal to $n_{max}/4$. Thus, the parameter expressing oxygen transport efficiency is closely related to the parameter expressing oxygen binding cooperativity. However, it should be stressed here that the values of P or S at which oxygen transport is maximized do not necessarily agree with those at which cooperativity is maximized [6].

We also analyzed the same OEC data as used in the present study using the two-state allosteric model of Monod *et al.* [7] instead of the Adair scheme, and obtained essentially the same results. The present method of analysis has the advantage that P_{dmax} can easily be evaluated from the inflection point of the usual OEC (S *vs.* P) and thus allowing the easy construction of the normalized OEC (S *vs.* P/P_{dmax}).

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